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Raw milk, allergies, asthma and
respiratory infections: main results of
the «PASTURE » international project.

Leche cruda, alergias, asma e
infecciones respiratorias: principales
resultados del proyecto internacional
«PASTURE».

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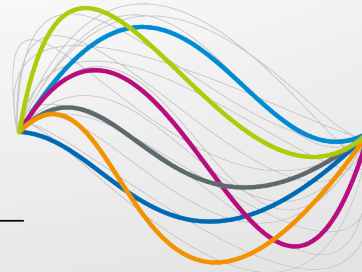


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L'essentiel & plus encore

Raw milk, allergies, asthma and respiratory infections: main results of the « PASTURE » international project

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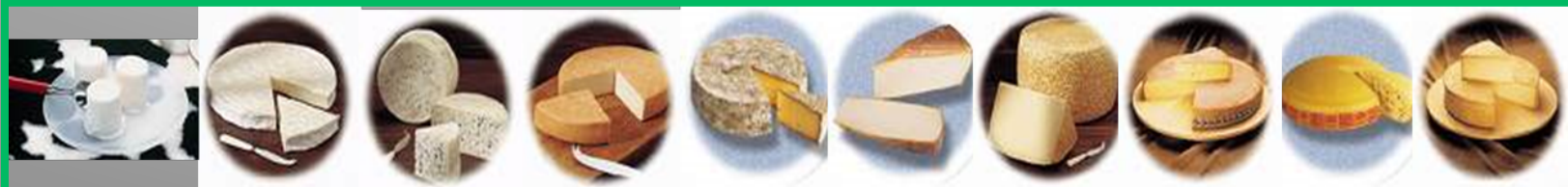
In memory to

Jean-Charles Dalphin,

On behalf of

Marie-Laure Dalphin, Amandine Divaret-Chauveau,

Sophie Nicklaus, Erika von Mutius

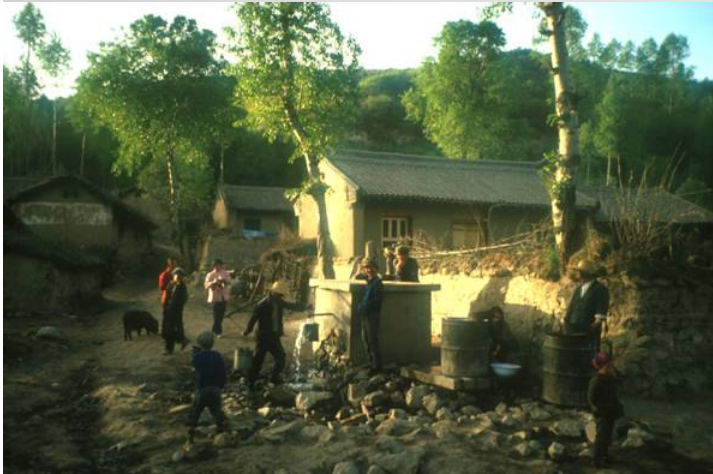


From 1960 to 2000: unprecedented increase in allergic/atopic diseases

significantly associated with

Urbanization
&
Development

Increased standard of
living



Decreased family size
Decreased number of infections in early
life:

bacteria and viruses: vaccination, antibiotics,
parasites: slaughtering and meat control...

Increased hygiene (**'hygiene' hypothesis**)
Major changes in feeding habits

Raw milk and allergy: a non-expected protective association...

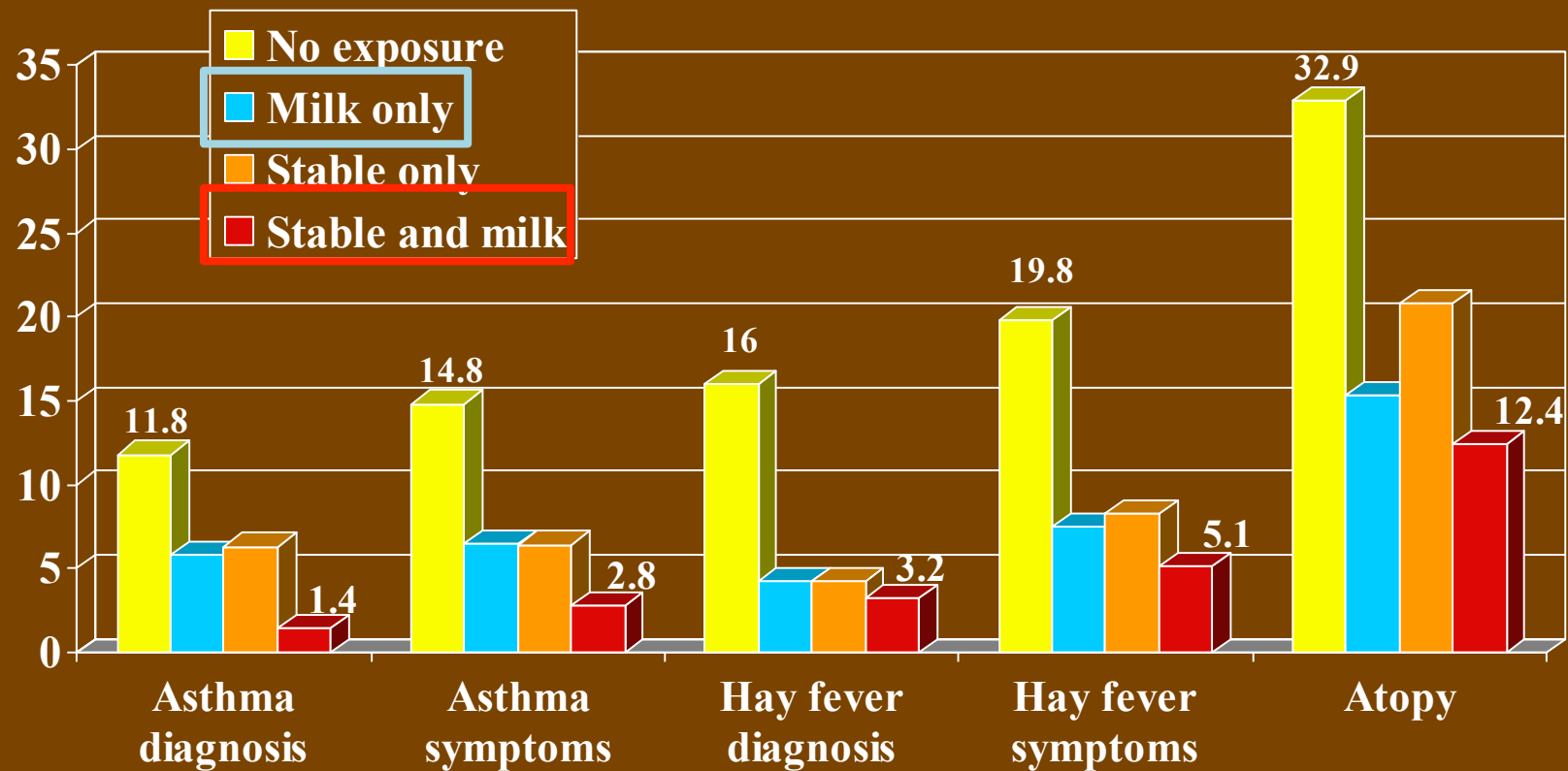
- Being born and living on a farm: major circumstances of protection against allergy
 - Time spent with animals, in the stable, in the barn
 - **Drinking raw milk**

(cf. Braun-Fahrländer and von Mutius, 2011; von Mutius, 2012)



- *for the pregnant woman and her child in early life*
- Association found in cross-sectional studies in many different countries
- **Also found in urban children drinking raw milk**

Observations in cross-sectional studies



The 'Alex' study

[Riedler et al, The Lancet, 2001]

The 'PASTURE cohort'

Longitudinal study of rural European children (from 2002)

- ✓ 500 born and living on a farm
- ✓ 500 not born and living on a farm

Follow-up from pregnancy to 17 yrs-old

1,000s of questionnaires on environment, diet, behaviour, occupation, health...

Blood, milk, and environmental samples

Medical visits at 1, 3, 4.5, 6, 10.5, 17 years

Microbiological, immunological, and genomic studies



Raw milk, raw milk-products, ... raw milk-cheeses

The PASTURE study did not specifically address the issue of raw milk-cheeses! Why??

- ✓ Studies based on correlations between
 - ✓ environmental and biological data (lab measurements),
 - ✓ medical data (questionnaires and medical visits), and
 - ✓ data collected by the parents (yearly questionnaires, 'first-year diary')

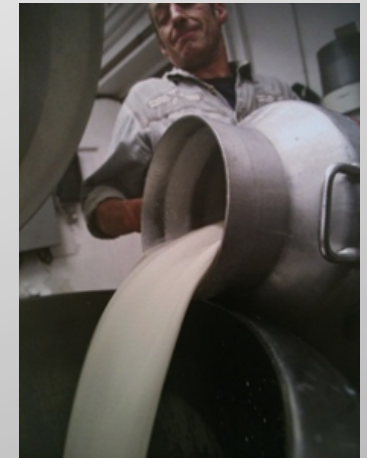


- ✓ Except for France, differentiation between the origin of the milk to prepare cheeses was judged non-reliable
- ✓ The question on 'farm-made cheese' did not cover all raw milk-cheeses (most are prepared outside the farm)

17 years later, the messages of PASTURE

Results of the PASTURE cohort confirm:

- *Contact with animals*
- *Stable and barn environment*
- *Drinking raw milk*



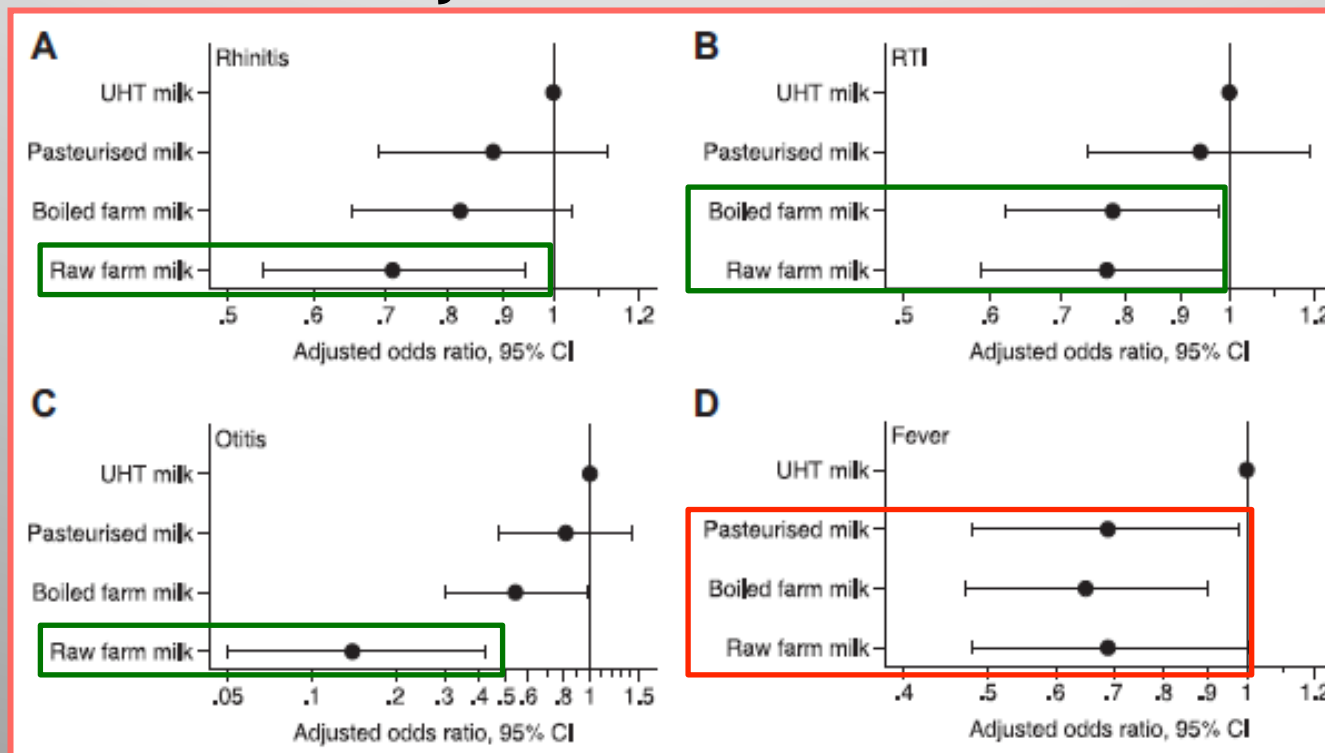
actually and independently protect against occurrence of allergic diseases later in life

- Atopic dermatitis
- Asthma
- Allergic rhinitis
- Allergic sensitization



17 years later, the messages of PASTURE

In addition, drinking raw milk protects against acute infections of the 1st year of life

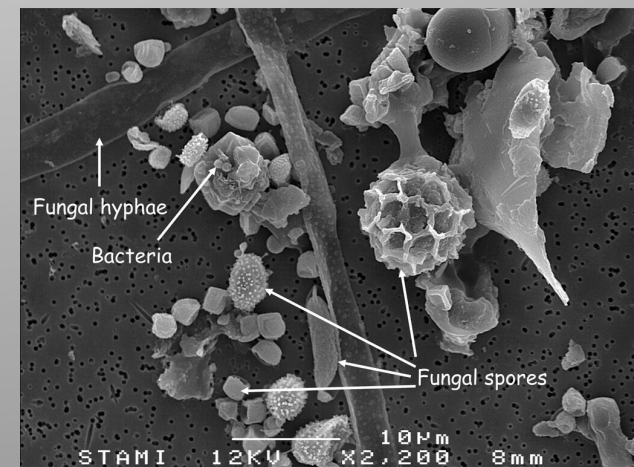


Correlations between type of milk and occurrence of infections in the 1st year of life [Loss et al, JACI, 2014]

1. Diversity matters!

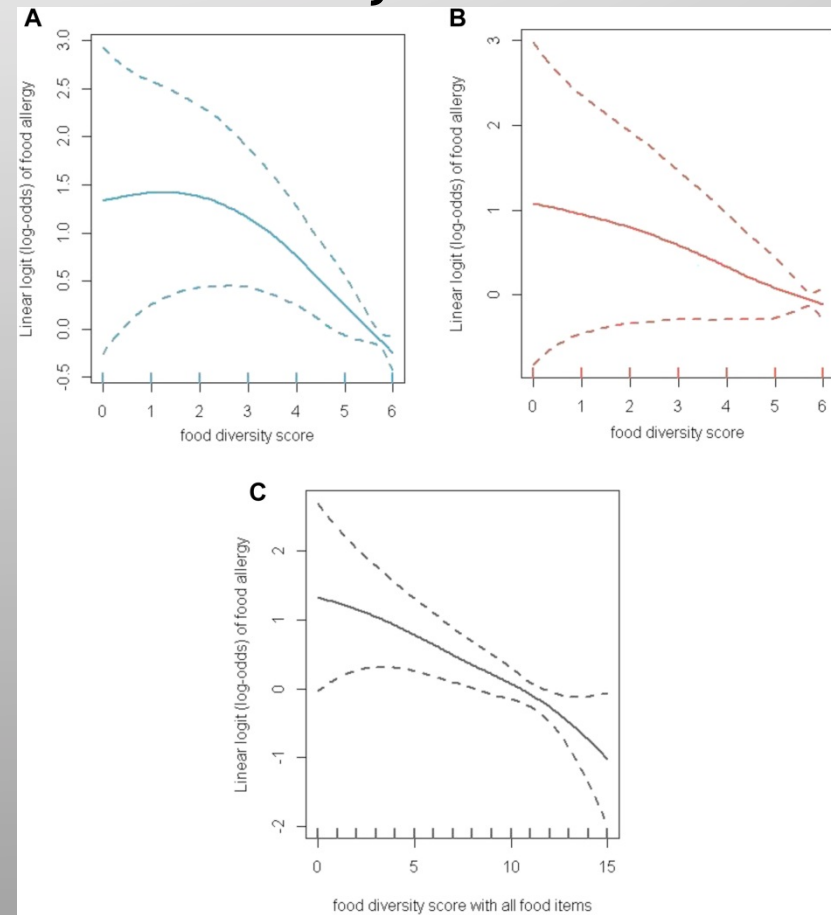
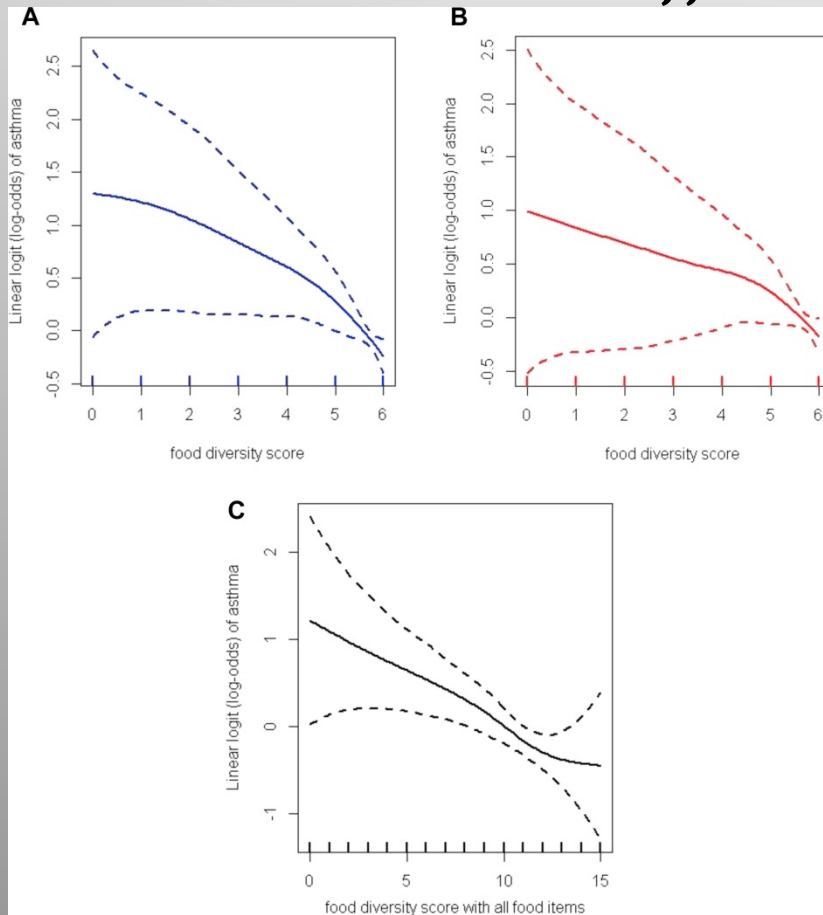
In the farm environment:

- Diversity of exposure
 - stable, barn, house
 - Microbes + pollen (hay), plant particles (straw)
- Diversity of animals
 - Farm animals ++, dogs and cats
- Diversity of micro-organisms
 - Gram positive et negative bacteria, actinomycetes,
 - Fungi: *Absidia* spp., *Eurotium* spp., *Cladosporium* spp., *Penicillium* spp.,
 - High diversity in traditional farms



1. Diversity matters!

- Early food diversification in the 1st year of life
Predictive effect of a food diversity score

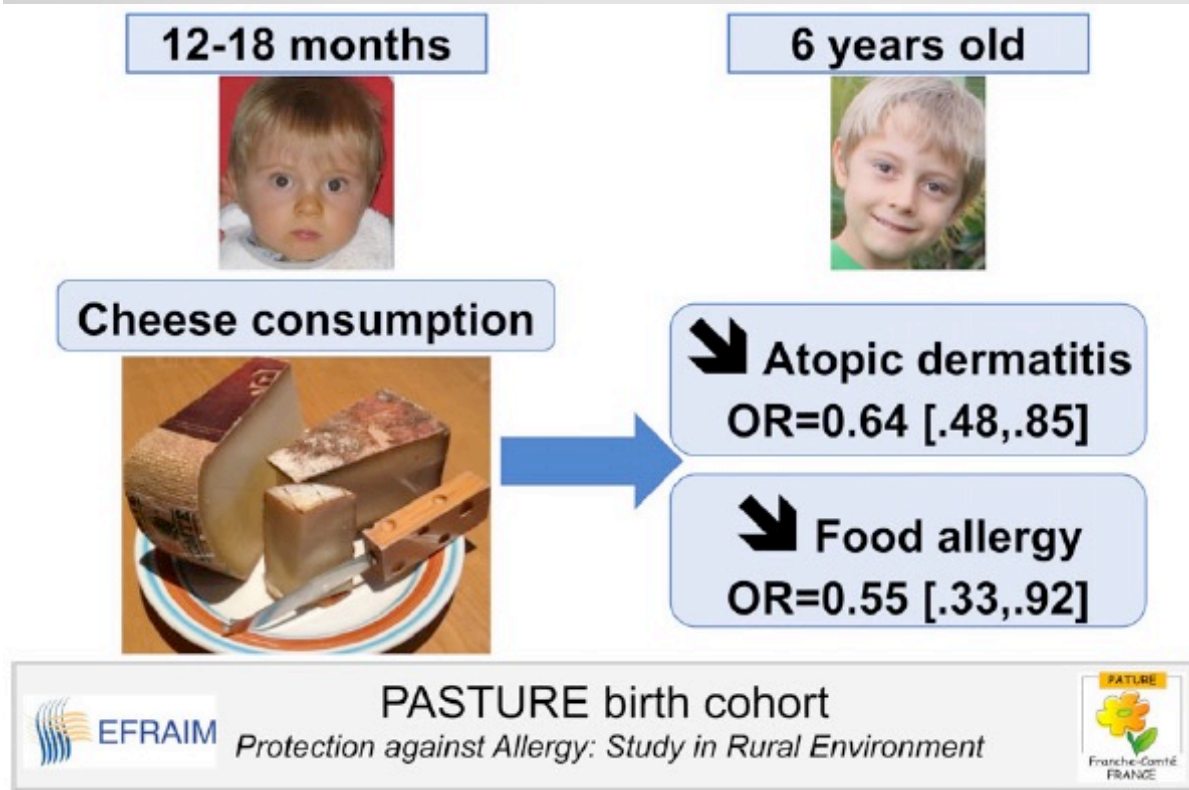


on asthma

[Roudit et al, JACI, 2014]

on food allergy

1. Both diversity and quantity matter... for cheeses



✓ Children who never or rarely ate cheese had higher risk of food allergy and allergic rhinitis

✓ Finnish children had the highest frequency of cheese consumption

✓ French children had the most diverse consumption of cheeses

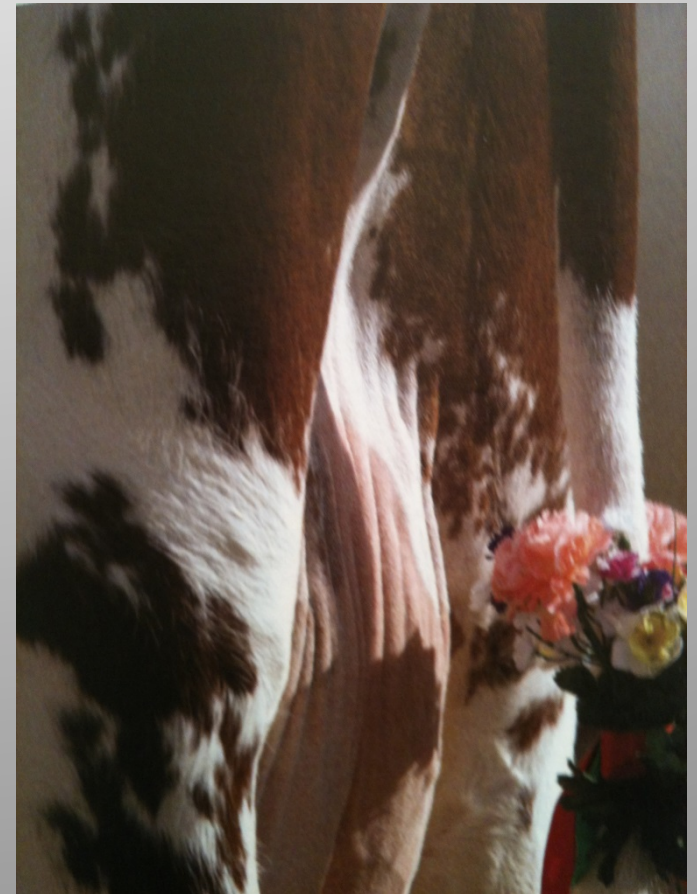
✓ Farmers' and non-farmers' children had similar cheese consumption

✓ Cheese consumption diversity was correlated with the mother's level of education

[Nicklaus et al, Allergy, 2018]

2. Raw milk : THE protective factor

- Most constantly found in analyses
- Independent of other ‘farm-associated’ factors
- Additional effect of ‘farm milk-’ butter, yoghurt, and cheese
- Mother’s consumption is also protective for her child
- Consumption in early life (< 6 years) is most important



2. Raw milk: THE protective factor

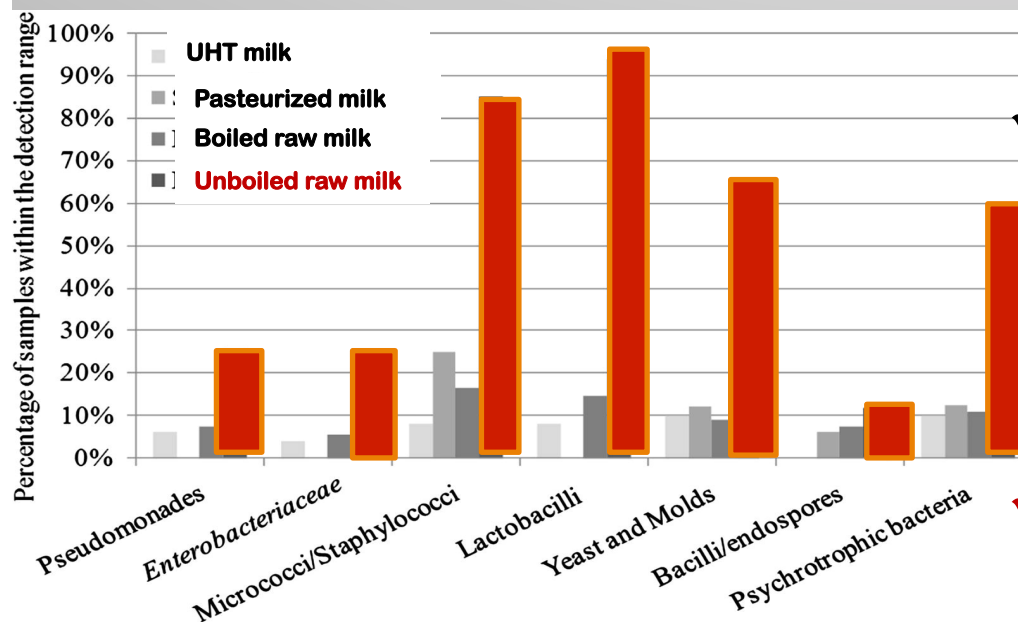
Role of the milk microflora?

The first hypothesis:

bacterial endotoxins

✓ No more endotoxins in the raw milk of the studied farms than in 'shop milk' kept by families

✓ Higher number and diversity of micro-organisms in raw milk



✓ No association between numbers of organisms (or single species) and clinical parameters (allergy, infection etc.)

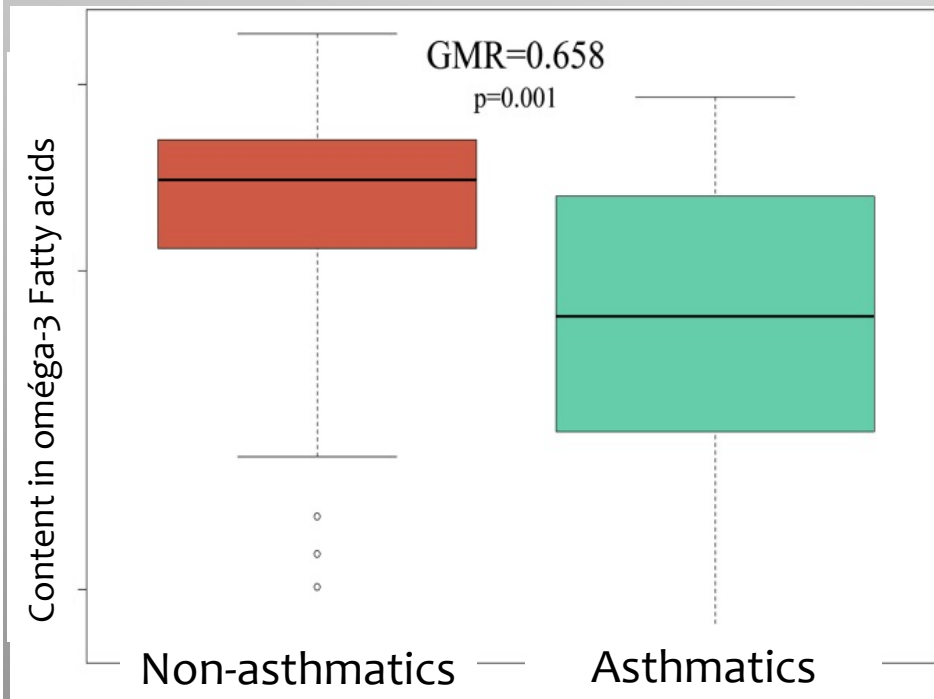
✓ Association with diversity (organisms similar to those found in the farm environment)

[Loss et al-GABRIELA. JACI, 2011]

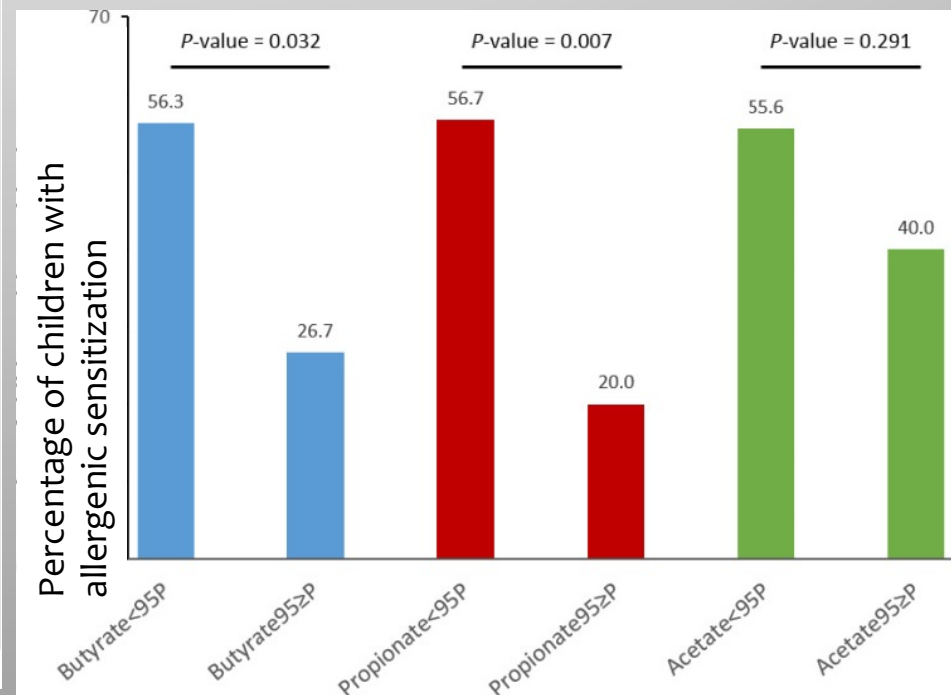
2. Raw milk: THE protective factor

Role of non-microbial components?

- ✓ **Whey proteins:** changes in α -lactalbumin, et β -lactoglobulin
- ✓ **Immunity components:** lactoferrin, IgA, TGF- β
- ✓ **Lipids:** Conjugated Linoleic Acids (CLA), Ω -3 Fatty Acids, Short Chain Fatty Acids



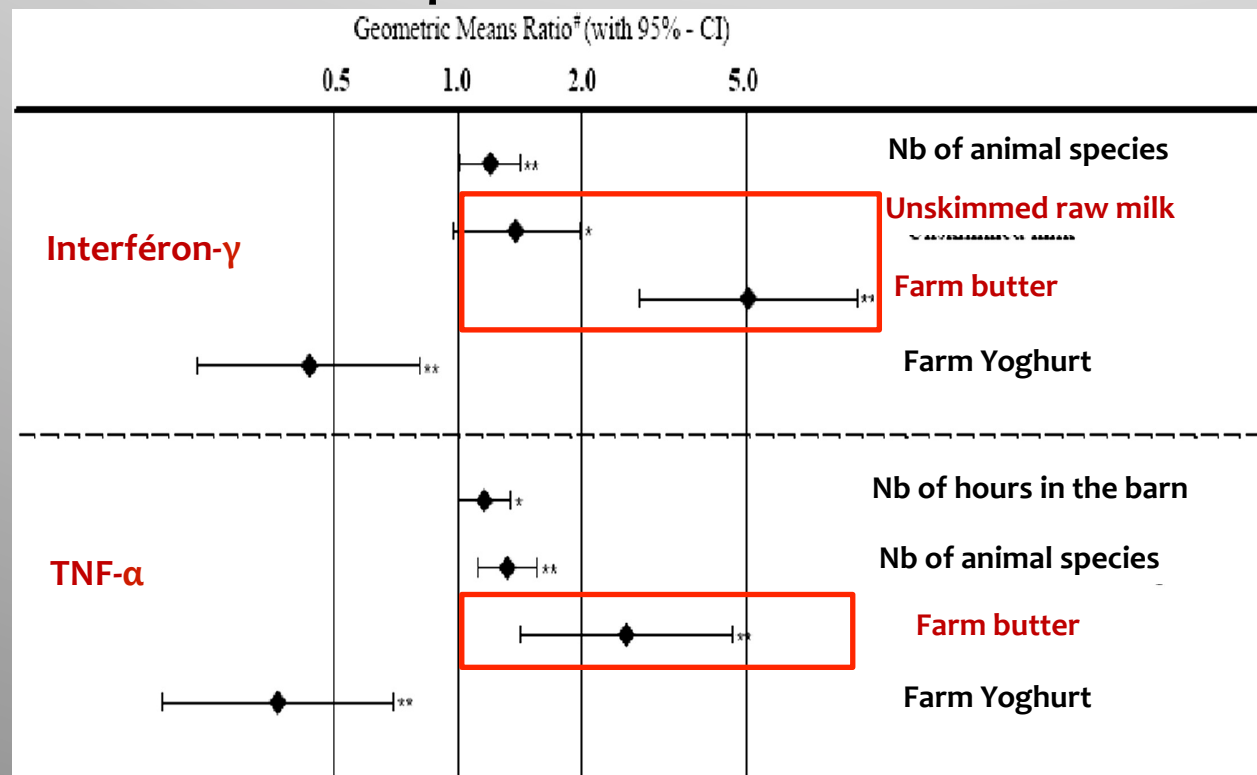
[Brick et al, JACI, 2016]



[Roudit et al, Allergy, 2018]

3. Early exposure is associated with immunological changes

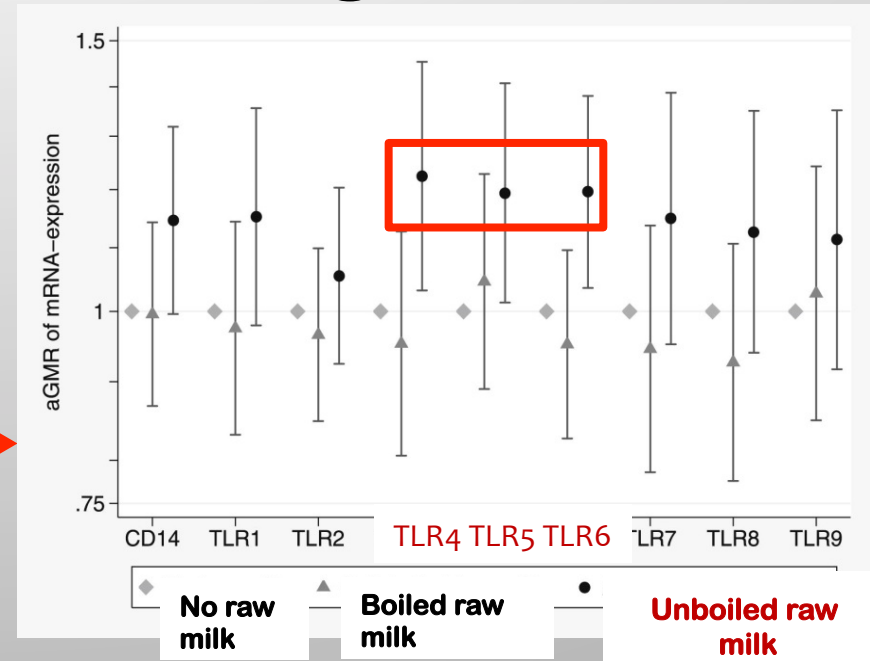
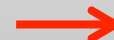
- Influence of **mother's exposure** to protective factors during pregnancy on immune response: true for raw milk and raw milk products



Production of cytokines by the immune cells of the cord blood, according to maternal exposure during pregnancy

3. Early exposure is associated with immunological changes

Influence of early raw milk consumption by the children on the development of 'innate' and 'adaptive' immunity

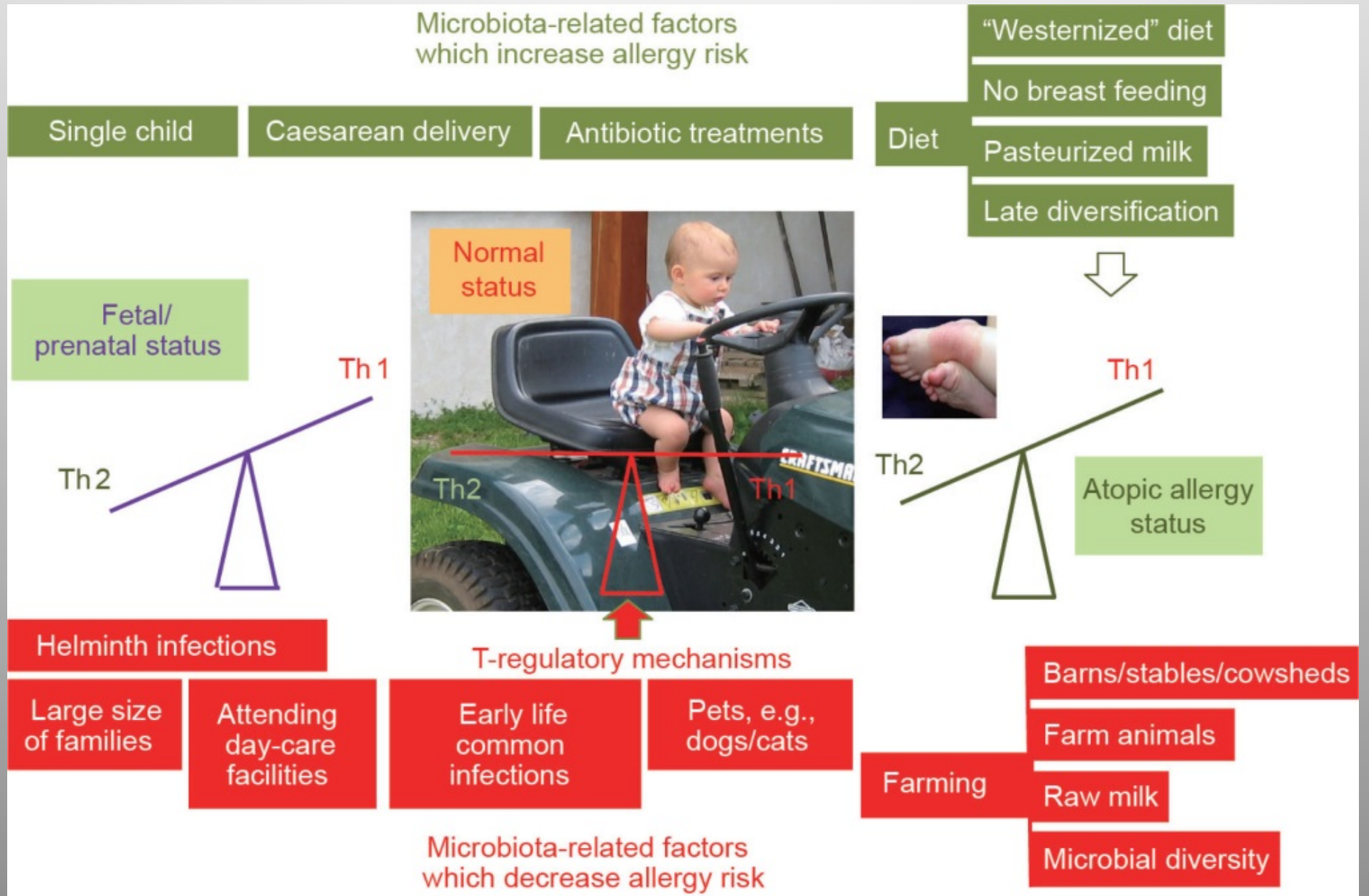


Influence on the expression of the Toll-like receptors [Loss et al. JACI, 2012]

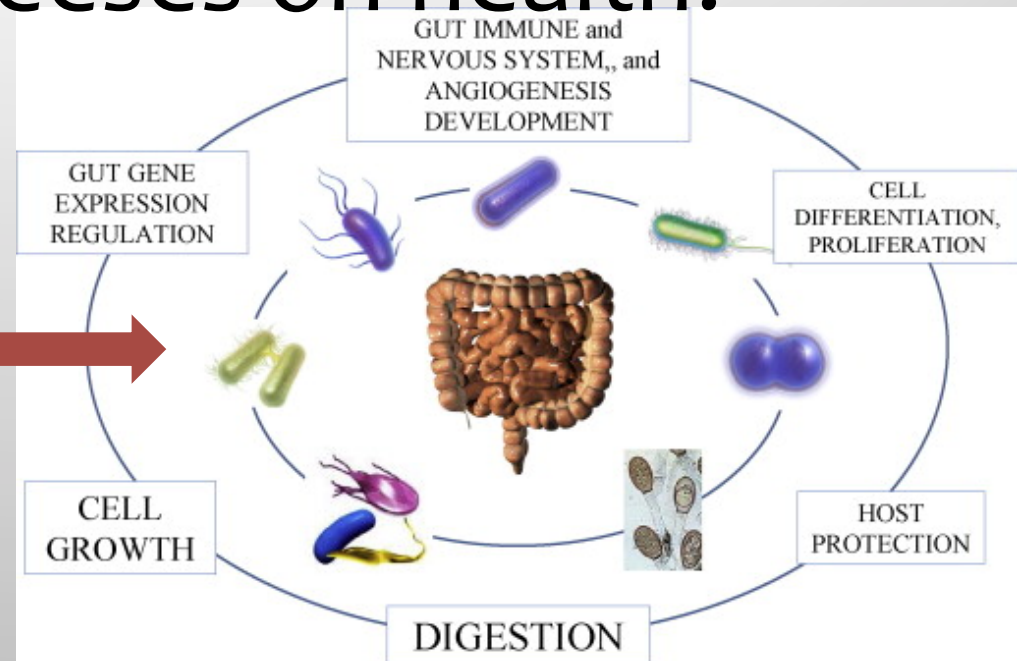
Stimulus	Marker	Farmers vs non-farmers	Stay in the stables	Raw milk consumption	Nb
PI	CD4 ⁺ CD25 ⁺	1.30 (1.11-1.54) .002‡	1.27 (1.08-1.50) .005‡	1.37 (1.17-1.61) <.001‡	258
	Upper 20% CD4 ⁺ CD25 ⁺ , FOXP3 ⁺	1.23 (1.04-1.46) .015	1.25 (1.05-1.48) .011‡	1.36 (1.15-1.60) <.001‡	258
LPS	CD4 ⁺ CD25 ⁺	1.32 (1.10-1.57) .003‡	1.27 (1.06-1.52) .010‡	1.41 (1.18-1.69) <.001‡	261
	Upper 20% CD4 ⁺ CD25 ⁺ , FOXP3 ⁺	1.44 (1.15-1.79) .001‡	1.35 (1.08-1.68) .008‡	1.57 (1.27-1.95) <.001‡	260

Influence on the development of T-regulator lymphocytes [Lluis et al. JACI, 2014]

The 'big picture'



Possible mechanisms of the effects of traditional cheeses on health?



Gut microflora ('microbiota'): at the centre of the immune system regulation as well as a variety of metabolisms

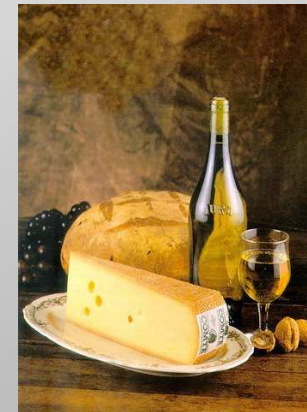
Intestinal microbiota:

- different in children living in 'developed' vs 'traditional' environment
- different in children with and without allergy;
- more diverse in 'protected rural children'

Confirmation from cohort studies [Azad, Clin Exp Allergy. 2015]

The Pasture study

- ✓ Confirmed the protective effect of raw milk consumed by the mother during pregnancy and by the children in the first 6 years of age
 - ✓ Against allergic disorders
 - ✓ Against acute infections of the first year of age
- ✓ Showed that this effect was increased by the child's consumption of farm dairy products for the first year of life and by the child's consumption of a variety of cheeses (including raw milk-cheeses +++) for the first 18 months
- ✓ Demonstrated an association with immunological parameters
- ✓ Suggested interference with genetic factors and intestinal and respiratory microbiota





Protection contre l'Allergie : Etude du milieu Rural et de son Environnement



L'essentiel
et plus encore



Thanks to the « PASTURE » team!

Prof. Jean-Charles Dalphin

- Dr Marie-Laure Dalphin
- Dr Vincent Kaulek
- Dr Jean-Jacques Laplante
- Dr Amandine Divaret-Chauveau
- Dr Gabriel Reboux
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- Dr Sophie Nicklaus
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- Nadia Guillou
- clinical research technicians and engineers
- Prof. Erika von Mutius and the country coordinators



And all 'PASTURE' families on board!



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